

# A meta-analysis of soil extracellular enzyme activities in response to global change

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## ABSTRACT

The crucial biogeochemical processes such as carbon and nutrient cycling are increasingly altered at the ecosystem scale by global environmental changes. Although soil extracellular enzyme activities (EEAs) play a critical role in biogeochemical processes, the global patterns of soil EEAs in a changing world remain elusive. Here, we synthesized eight EEAs involved in carbon (C), nitrogen (N) and phosphorus (P) acquisition in response to seven global change factors based on 132 peer-reviewed papers. Our results showed that elevated CO<sub>2</sub> concentration had no significant effects on soil EEAs. Nitrogen addition stimulated C-acquisition (9.1%) and P-acquisition (9.9%) EEAs, but suppressed oxidase activity (−6.8%). Phosphorus addition decreased P-acquisition EEA (−19.8%), while combined N and P addition increased C-acquisition EEA (30.7%). Moreover, decrease in precipitation dramatically suppressed oxidase activity (−47.2%), increase in precipitation marginally stimulated N-acquisition EEA (16.7%), while warming significantly decreased oxidase activity (−10.9%) and had minor positive effect on hydrolytic enzymes. Overall, our results provide some evidence (with exceptions) for the resource allocation theory of microbial enzyme production, and indicate that EEAs are generally more sensitive to nutrient addition than to atmospheric and climate change. We have shown that global environmental changes can alter EEAs, which have implications for soil carbon storage, nutrient cycling, and plant productivity. Further research is needed to elucidate the underlying mechanisms driving the responses of EEAs to global change and to collect data from particularly non-forest ecosystems (e.g., wetland, tundra and desert) and global-change drivers (other than N addition) that lack of EEA data. Our synthesis of the responses of soil enzyme activities to global-change drivers can be used to develop better representations of microbial processes in ecosystem and earth system models.

## 1. Introduction

Human-induced global change including elevated CO<sub>2</sub> concentration, atmospheric nitrogen (N) and phosphorus (P) deposition, global warming, and altered precipitation has dramatically altered carbon (C) and nutrient cycling in terrestrial ecosystems. For instance, the annual N deposition is predicted to increase two or threefold in the future (Galloway et al., 2004) and this enhanced N availability can alter microbial community structure and activity, which has close connection with C and nutrient cycling (Janssens et al., 2010; Cusack et al., 2011; Leff et al., 2015). In the past decades, numerous field manipulation experiments have been well established worldwide to investigate the response of terrestrial ecosystems to global change. As a molecular protein that microbes produce to excavate nutrients for plant and their

own growth, soil extracellular enzyme plays a critical role in biogeochemical processes as mediating the degradation, transformation and mineralization of soil organic matter (Sinsabaugh, 2010; Burns et al., 2013). In consequence, soil extracellular enzyme activities (EEAs) have gradually received attention and become an increasingly common tool for indicating microbial response to global change in field manipulation experiments (Weedon et al., 2011; Henry, 2012; Burns et al., 2013). These experiments were conducted in diverse ecosystems from arctic tundra to tropical forest, and various responses (positive, negative, neutral) of EEAs to nutrient or resource (C, N and P) addition and climate change (warming and altered precipitation) have been observed across different ecosystems globally (Henry et al., 2005; Allison and Treseder, 2008; Keeler et al., 2009; Turner and Wright, 2014; Meier et al., 2015).

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The responses of soil EEAs to nutrient or resource manipulation are commonly explained by the “resource allocation theory” (Sinsabaugh and Moorhead, 1994; Allison and Vitousek, 2005) which expresses that soil microbes regulate enzyme production based on the availability of resources. This microbial economic theory predicts that microbes would change their EEAs as a survival strategy – increasing EEAs in the presence of complex resource and decreasing EEAs when simple nutrient like inorganic nitrogen and assimilable carbon are available (Sinsabaugh and Moorhead, 1994; Allison and Vitousek, 2005). This dynamic pattern has been demonstrated in incubation and greenhouse experiments (Allison and Vitousek, 2005; Stone et al., 2013) and also been observed in many field experiments. For instance, simple nutrients addition like inorganic N fertilizers are widely observed to stimulate phosphatase activity while inorganic P fertilizers consistently suppress it (Marklein and Houlton, 2012; Turner and Wright, 2014; Yokoyama et al., 2017). Moreover, elevated CO<sub>2</sub> could enhance belowground carbon input like dead roots and root exudates, which are considered as “complex nutrient” addition, and result in stimulation of C-acquisition EEA (Finzi et al., 2006; Meier et al., 2015). However, results inconsistent with this theory are also reported in previous studies (Saiya-Cork et al., 2002; Zeglin et al., 2007; Keeler et al., 2009; Dong et al., 2015; Jing et al., 2017).

The responses of soil EEAs to climate change (warming and altered precipitation) are dependent on the effects of climate change on soil properties, such as soil temperature and moisture, and availability of labile C, N and P resources (Henry, 2012). Soil warming can increase enzyme production if it enhances plant substrate input, but can also stimulate enzyme stabilization and turnover, and thus have an uncertain effect on the enzyme pool size which is the potential EEA measured under controlled conditions and common temperature in the lab (Henry, 2012; Burns et al., 2013). Additionally, soil moisture has been recognized as an important control of EEA at regional scale (Brockett et al., 2012). Increase in soil moisture should result in higher EEA, until the soil becomes anaerobic and substrate diffusion or oxygen content limits EEA (Henry, 2012). Therefore, water addition generally stimulated EEA, while rainfall exclusion or warming-induced drought often suppressed EEA (Allison et al., 2008; Kreyling et al., 2008; Sardans and Penuelas, 2010; Zhou et al., 2013). However, climate change also affects soil nutrient availability and other properties such as soil pH and microbial community, which may confound the direct effect of temperature and moisture on soil EEAs.

Given that soil EEAs were mediated by multiple factors (resource availability, environmental condition, and microbial community), the responses of EEAs to global change drivers remain poorly understood. In addition to numerous field experiments which measured soil EEAs in response to various global change drivers (Saiya-Cork et al., 2002; Henry et al., 2005; Finzi et al., 2006; McDaniel et al., 2013; Meier et al., 2015; Jing et al., 2017), a number of meta-analysis studies have synthesized the impact of global change on soil EEAs. For example, Kelley et al. (2011) showed that only chitinase activity consistently increased under elevated CO<sub>2</sub>. Marklein and Houlton (2012) reported that phosphatase activity was enhanced by N addition, but suppressed by P addition. Jian et al. (2016) found that hydrolytic enzymes were enhanced, but oxidative enzymes were suppressed by N addition. Moreover, Henry (2012) and Ren et al. (2017) reviewed the responses of soil EEAs to climate change (warming and altered precipitation). These reviews and syntheses have greatly improved our understanding of soil EEAs to global change drivers. However, these studies used different criteria in their selection of data (field vs. lab and greenhouse manipulations, natural vs. agricultural ecosystems, C vs. N and P acquisition enzymes), and many field studies on soil EEAs in response to global change drivers have been published (including those published in Chinese literature) in the last 3–5 years.

Therefore, as a further development of these previous syntheses (Kelley et al., 2011; Henry, 2012; Marklein and Houlton, 2012; Jian et al., 2016; Ren et al., 2017), we performed a comprehensive updated

global meta-analysis of the responses of soil EEAs to various global change drivers. We included six hydrolytic and two oxidative enzymes involved in C, N and P acquisition that are commonly measured, and seven global change drivers related to resource manipulation and climate change (elevated CO<sub>2</sub>, N addition, P addition, combined N and P addition, decrease and increase in precipitation, and warming). Based on previous experimental and synthesis studies, we hypothesized that: (1) elevated CO<sub>2</sub> would stimulate EEAs because complex nutrients (plant-derived carbon substrates) were added, but N and P addition (simple inorganic nutrients) would suppress enzymes acquiring the added nutrients and enhance enzymes acquiring other nutrients; and (2) warming and increase in precipitation could increase EEAs, while decrease in precipitation could decrease EEAs.

## 2. Materials and methods

### 2.1. Data collection

Database in this meta-analysis was compiled by using Web of Science and CNKI (China National Knowledge Infrastructure) search engine (1900–2017). A total of seven experimental treatments were considered as global change factors related to nutrient addition and climate change. These treatments include CO<sub>2</sub> enrichment, nitrogen addition, phosphorus addition, combined nitrogen and phosphorus addition, precipitation decrease, precipitation increase and temperature increase. Additionally, data of eight soil enzymes and four soil properties were compiled from both control and treatment in these experiments. The eight enzymes include six hydrolytic enzymes, namely  $\beta$ -1,4-glucosidase (BG),  $\beta$ -D-cellobiohydrolase (CB),  $\beta$ -1,4-N-acetyl-glucosaminidase (NAG), leucine amino peptidase (LAP), urease, and acid phosphatase (AP), and two oxidative enzymes, phenol oxidase (POX) and peroxidase (PER) (Table S1). The four soil properties, including pH, soil organic carbon (SOC), microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN), are only extracted from the papers containing EEAs and global-change treatments.

Proper papers were selected by meeting the following criteria: (1) only field experiments in natural terrestrial ecosystems were selected, while cropland, greenhouse and lab-incubation experiments were not included; (2) only corresponding nutrients (N and/or P) were added in the nutrient addition experiments (no other micronutrients were added); (3) the means, sample sizes, standard errors or standard deviations were reported; (4) if data from the same experiment were reported more than once at different time, only the latest data were selected; (5) data from only the surface soil layer were included; and (6) data were extracted either directly from tables and text, or indirectly by using software Engauge Digitizer from figures. In addition, experiment location, soil type, sampling depth and vegetation type were also extracted.

In total, 132 published papers (Appendix) meeting the criteria were selected for this study, including 1577 individual observations and 133 different sites mainly distributed over North America, Europe and East Asia (Fig. 1). Among the seven global change factors, N addition had the most data with 1056 individual observations from 70 different sites (Table 1, Fig. 1), while other global change factors were limited to < 200 observations from < 30 sites globally. As such, we further categorized data of N-addition experiments into four groups following similar work (Zhou et al., 2014; Jian et al., 2016): 1) ecosystem type (forest, grassland, tundra, wetland and desert); 2) fertilizer type (only NH<sub>4</sub><sup>+</sup>, only NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub>NO<sub>3</sub> and urea); 3) application rate (ranging from 5 to 600 kg N ha<sup>-1</sup> yr<sup>-1</sup>), which were divided into low ( $\leq 50$  kg N ha<sup>-1</sup> yr<sup>-1</sup>), mid ( $> 50$  and  $\leq 100$  kg N ha<sup>-1</sup> yr<sup>-1</sup>) and high ( $> 100$  kg N ha<sup>-1</sup> yr<sup>-1</sup>) levels; and 4) treatment duration (ranging from < 1 to 19 years), which were divided into short ( $\leq 3$  years), mid ( $> 3$  and  $\leq 10$  years) and long ( $> 10$  years) terms.

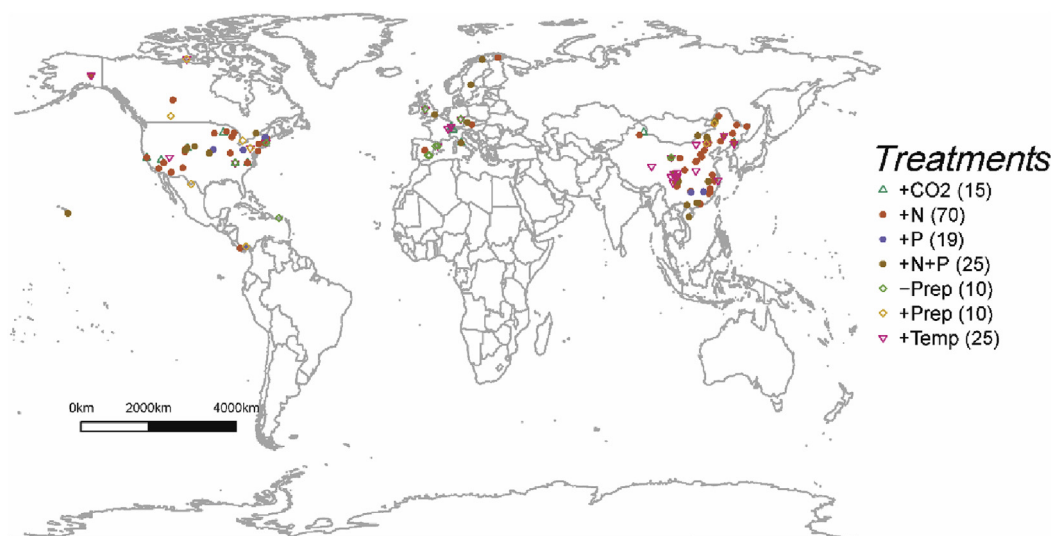


**Fig. 1.** Global distribution of the sites used in this meta-analysis. Numbers in parentheses are the number of sites for each global-change treatment. +CO<sub>2</sub>, +N, +P, +N + P, -Prep, +Prep, +Temp represent CO<sub>2</sub> enrichment, nitrogen addition, phosphorus addition, combined nitrogen and phosphorus addition, precipitation decrease, precipitation increase, and temperature increase (warming), respectively.

## 2.2. Data analysis

To examine the effects of global change factors on soil enzyme activity, the natural log-transformed response ratio ( $\ln RR$ ) was used as an index, defined as the “effect size”, to weigh the effects (Hedges et al., 1999). The effect size of individual observation ( $d_{ij}$ ) was calculated as the natural log-transformed ratio of its value in the treatment group ( $X_e$ ) to the value in the control group ( $X_c$ ):

$$d_{ij} = \ln RR_{ij} = \ln(X_e/X_c) = \ln X_e - \ln X_c$$

The variance ( $v$ ) of individual observation was calculated as:

$$v_{ij} = \frac{S_e^2}{n_e X_e^2} + \frac{S_c^2}{n_c X_c^2}$$

$S_e$ ,  $S_c$ ,  $n_e$ ,  $n_c$ ,  $X_e$  and  $X_c$  represented the standard deviations ( $S$ ), sample sizes ( $n$ ) and mean values ( $X$ ) for the treatment ( $e$ ) and the control ( $c$ ) group, respectively.

The weight factor ( $w_{ij}$ ) of individual observation was calculated as the inverse of the variance ( $v_{ij}$ ):

$$w_{ij} = 1/v_{ij}$$

In order to estimate the overall effects of treatment group to the control group, the “mean effect size” ( $E_{++}$ ) was calculated as:

$$E_{++} = \frac{\sum_{i=1}^m \sum_{j=1}^{n_i} w_{ij} d_{ij}}{\sum_{i=1}^m \sum_{j=1}^{n_i} w_{ij}}$$

$n$  represented the number of observations in every corresponding compared group and  $m$  represented the number of groups.

Percentage values transformed from the mean effect size were also used to express the effects of different treatments (Table 2):

$$\text{Percentage}(\%) = [\exp(E_{++}) - 1] \times 100\%$$

All the calculation was performed by using Meta-Win software (2.1)

**Table 1**

The number of papers, observations and sites used in this meta-analysis.

|                 | + CO <sub>2</sub> | +N   | +P | +N + P | -Prep. | + Prep. | + Temp. | Total <sup>a</sup> |
|-----------------|-------------------|------|----|--------|--------|---------|---------|--------------------|
| Journal article | 18                | 71   | 15 | 20     | 9      | 12      | 26      | 132                |
| Observation     | 53                | 1056 | 83 | 130    | 40     | 46      | 169     | 1577               |
| Site            | 15                | 70   | 19 | 25     | 10     | 10      | 25      | 133                |

<sup>a</sup> Journal articles and sites containing several global-change treatments were only counted once.

(Rosenberg et al., 2000). A random effects model was used to determine whether the effects of different global change factors were significant or not. Results of bootstrapping tests with 999 iterations were used to generate the 95% confidence interval (CI) (Adams et al., 1997; Hedges et al., 1999). When the 95% CI value of the mean effect size did not overlap with zero, the effects of global change factors were considered to be significant ( $P < 0.05$ ). Publication bias (Table S2) was tested with rank correlations between individual effect sizes and their variations (Dieleman and Janssens, 2011).

A continuous randomized effects model in Meta-Win was applied to estimate the relationship between the effect size ( $\ln RR$ ) and the application rate and treatment duration of N addition (Table S3). Statistical results were reported as total heterogeneity among all studies ( $Q_T$ ), heterogeneity among group cumulative effect sizes ( $Q_M$ ) and residual error ( $Q_E$ ) (Rosenberg et al., 2000).

## 3. Results

### 3.1. Effects of nutrient-addition treatments (C, N, and P addition)

Elevated CO<sub>2</sub> tended to stimulate hydrolytic enzyme activities, but partly due to the small sample size ( $< 12$ ), the effect was not statistically significant ( $P > 0.05$ , Fig. 2, Table 2). For example, the activity of NAG, a key enzyme for N mineralization, was marginally increased by 12.9% with elevated CO<sub>2</sub> ( $P < 0.10$ ). When we grouped the enzymes into functional groups (C-, N- and P-acquisition hydrolytic and oxidative enzymes), the effect of elevated CO<sub>2</sub> was still not significant ( $P > 0.05$ , Fig. 4, Table 2).

N addition significantly increased BG (10.8%) and AP (9.9%) activity ( $P < 0.05$ ), decreased POX (−9.3%) activity ( $P < 0.05$ ), but had minor effect on other enzymes ( $P > 0.05$ , Fig. 2, Table 2). When we grouped the enzymes into functional groups, the effect of N addition was similar – it stimulated C- and P-acquisition enzymes (9.1% and

**Table 2**

Summary of the relative change of microbial enzymes and soil properties under different global change factors. Bold numbers represented significant changes when the 95% confidence interval (CI) of the effect size did not overlap with zero. Abbreviations of the enzymes and soil properties are:  $\beta$ -1,4-glucosidase (BG),  $\beta$ -D-cellobiohydrolase (CB),  $\beta$ -1,4-N-acetyl-glucosaminidase (NAG), leucine amino peptidase (LAP), urease, acid phosphatase (AP), phenol oxidase (POX), peroxidase (PER), soil organic carbon (SOC), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN).

|                       | +CO <sub>2</sub> | +N           | +P            | +N + P        | -Prep.        | + Prep.      | + Temp.       |
|-----------------------|------------------|--------------|---------------|---------------|---------------|--------------|---------------|
| BG                    | 4.2%             | <b>10.8%</b> | 12.5%         | <b>45.9%</b>  | −4.6%         | −1.0%        | 0.1%          |
| CB                    | 11.1%            | 5.4%         | −4.3%         | −15.0%        | NA            | 14.8%        | 11.7%         |
| NAG                   | 12.9%            | 2.0%         | −4.1%         | <b>−18.3%</b> | 29.5%         | <b>25.2%</b> | 10.9%         |
| LAP                   | NA               | 5.1%         | 10.5%         | −13.4%        | NA            | <b>24.5%</b> | 1.4%          |
| Urease                | 1.5%             | −2.8%        | NA            | <b>32.7%</b>  | −30.6%        | NA           | 2.4%          |
| AP                    | −1.8%            | <b>9.9%</b>  | <b>−19.8%</b> | −13.2%        | −5.1%         | 8.6%         | 7.1%          |
| POX                   | −10.7%           | <b>−9.3%</b> | −16.4%        | −28.4%        | <b>−47.2%</b> | 42.8%        | −8.4%         |
| PER                   | NA               | −4.0%        | <b>11.1%</b>  | −5.0%         | NA            | NA           | <b>−8.6%</b>  |
| C-acquisition enzymes | 8.5%             | <b>9.1%</b>  | 10.1%         | <b>30.7%</b>  | −4.6%         | 0.0%         | 4.0%          |
| N-acquisition enzymes | 6.7%             | −0.5%        | 2.6%          | −4.5%         | −17.6%        | 16.7%        | 3.3%          |
| P-acquisition enzymes | −1.8%            | <b>9.9%</b>  | <b>−19.8%</b> | −13.2%        | −5.1%         | 8.6%         | 7.1%          |
| Oxidase               | −10.7%           | <b>−6.8%</b> | −7.3%         | −21.6%        | <b>−47.2%</b> | 42.8%        | <b>−10.9%</b> |
| pH                    | NA               | <b>−3.7%</b> | <b>2.2%</b>   | NA            | NA            | NA           | −0.2%         |
| SOC                   | NA               | <b>3.6%</b>  | 0.1%          | <b>8.4%</b>   | NA            | NA           | 3.5%          |
| MBC                   | NA               | −7.0%        | NA            | NA            | NA            | 15.3%        | 1.9%          |
| MBN                   | 8.3%             | <b>14.2%</b> | NA            | NA            | NA            | NA           | 5.2%          |

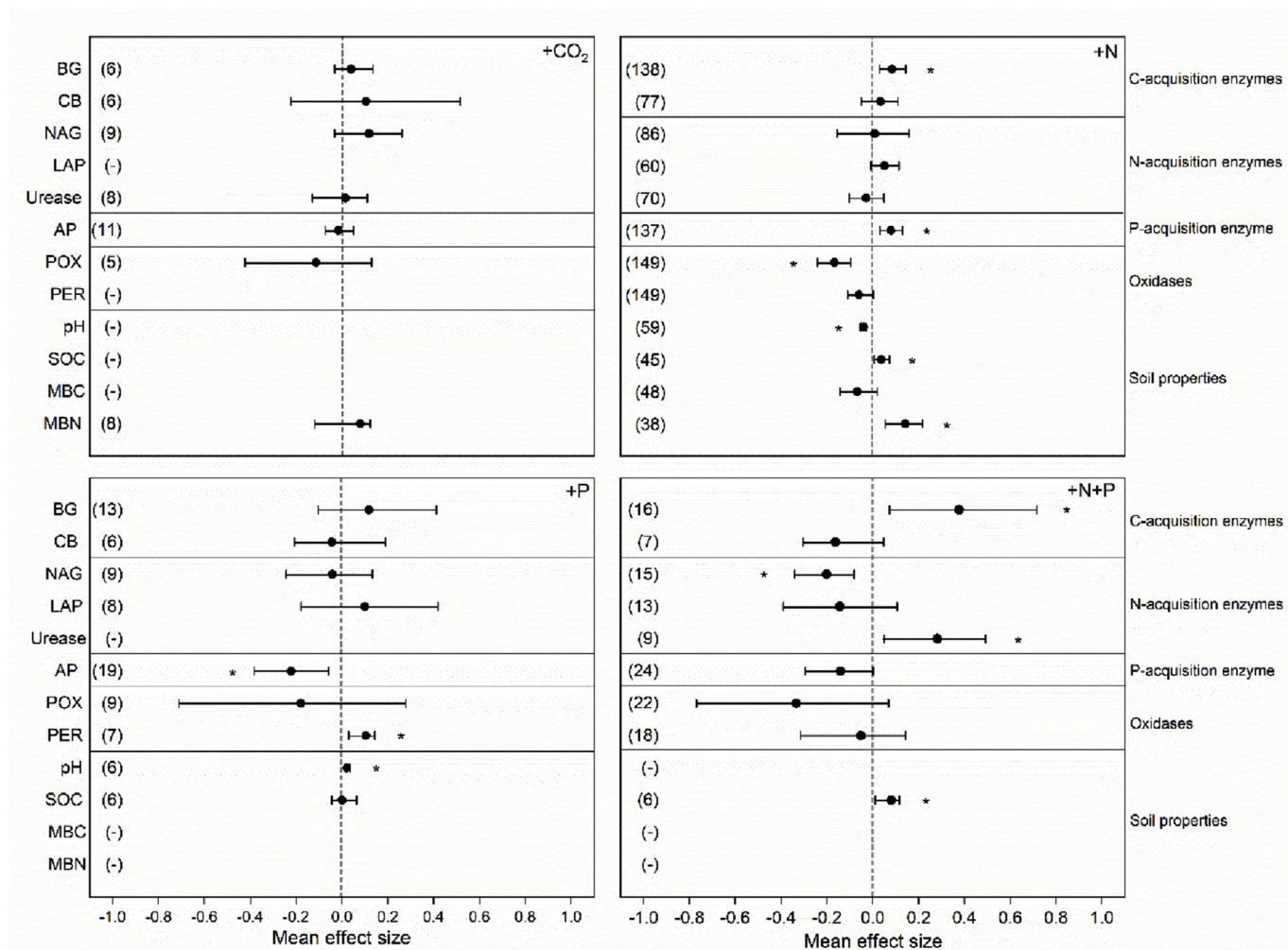


**Fig. 2.** Mean effect sizes of four global-change treatments (CO<sub>2</sub>, N, P and NP addition) on eight soil enzymes and four soil properties. The number in parentheses represents the sample size for each variable, and error bars represent 95% confidence intervals (CIs). \* means the mean effect size is significantly different with zero (P < 0.05).

9.9% respectively,  $P < 0.05$ ), suppressed oxidative enzymes ( $-6.8\%$ ), but had minor effect on N-acquisition enzymes ( $P > 0.05$ , Fig. 4, Table 2). Moreover, N addition led to significant soil acidification ( $P < 0.05$ ), higher SOC ( $3.6\%$ ,  $P < 0.05$ ) and MBN ( $14.2\%$ ,  $P < 0.05$ ), and minor decrease in MBC ( $P > 0.05$ , Fig. 2, Table 2).

P addition significantly decreased AP ( $-19.8\%$ ) activity ( $P < 0.05$ ), increased PER ( $11.1\%$ ) activity ( $P < 0.05$ ), but had no significant effect on other enzymes ( $P > 0.05$ , Fig. 2, Table 2). When we grouped the enzymes into functional groups, P addition only suppressed P-acquisition enzymes ( $-19.8\%$ ,  $P < 0.05$ ), with little effect on other enzymes ( $P > 0.05$ , Fig. 4, Table 2). Additionally, P addition increased soil pH ( $P < 0.05$ , Fig. 2, Table 2).

The combined N and P addition significantly increased BG ( $45.9\%$ ) and urease ( $32.7\%$ ) activity ( $P < 0.05$ ), decreased NAG ( $-18.3\%$ ) activity ( $P < 0.05$ ), but had no significant effect on other enzymes ( $P > 0.05$ , Fig. 2, Table 2). When we grouped the enzymes into functional groups, NP addition only enhanced C-acquisition enzymes ( $30.7\%$ ,  $P < 0.05$ ), but had no effect on other enzymes ( $P > 0.05$ , Fig. 4, Table 2). In addition, NP addition increased SOC by  $8.4\%$  ( $P < 0.05$ , Fig. 2, Table 2).

### 3.2. Effects of climate-change treatments (temperature and precipitation manipulation)

Decrease in precipitation (drought) significantly suppressed urease ( $-30.6\%$ ) and POX ( $-47.2\%$ ) activity ( $P < 0.05$ ), but had no significant effect on other enzymes ( $P > 0.05$ , Fig. 3, Table 2). For functional groups of enzymes, drought only significantly suppressed oxidase activity ( $-47.2\%$ ,  $P < 0.05$ ), but had insignificant, negative effect on other nutrient-acquisition enzymes ( $P > 0.05$ , Fig. 4, Table 2).

Increase in precipitation significantly stimulated NAG ( $25.2\%$ ) and LAP ( $24.5\%$ ) activity ( $P < 0.05$ ), but have no significant effect on other enzymes ( $P > 0.05$ , Fig. 3, Table 2). For functional groups of enzymes, the positive effect of drought on these enzymes became insignificant ( $P > 0.05$ , Fig. 4, Table 2).

Warming tended to stimulate hydrolytic enzymes, although the effect was not significant ( $P > 0.05$ , Fig. 3, Table 2). In contrast, warming suppressed oxidative enzymes, particularly PER ( $P < 0.05$ , Fig. 3, Table 2). For functional groups of enzymes, the pattern was similar – positive but insignificant effect on C, N, and P-acquisition hydrolytic enzymes, but significant negative effect on oxidase ( $P < 0.05$ , Fig. 4, Table 2). Moreover, warming had minor effect on soil properties ( $P > 0.05$ , Fig. 3, Table 2).

### 3.3. Comparison between forest and grassland ecosystems

Among the five ecosystems in this study (forest, grassland, tundra, wetland and desert), forest and grassland had the most data. Therefore, we compared the responses of four representative enzymes (BG, NAG, AP and POX) to global-change drivers in these two ecosystems (Fig. 5).

For BG (a representative C-acquisition enzyme) activity, the seven global-change drivers had no significant impact in forest or grassland ecosystems ( $P > 0.05$ , Fig. 5), except that N addition stimulated BG activity in forest (but not in grassland) ecosystem ( $P < 0.05$ , Fig. 5).

For NAG (a representative N-acquisition enzyme) activity, it was suppressed by NP addition in both forest and grassland ecosystems ( $P < 0.05$ , Fig. 5), stimulated by elevated  $\text{CO}_2$ , increase in precipitation, and warming in grassland ecosystem ( $P < 0.05$ , Fig. 5), and showed no response to other global-change drivers ( $P > 0.05$ , Fig. 5).

The P-acquisition enzyme (AP) activity was suppressed by P addition in both forest and grassland ecosystems ( $P < 0.05$ , Fig. 5), stimulated by N addition in forest ecosystem ( $P < 0.05$ , Fig. 5), and showed no response to other global-change drivers ( $P > 0.05$ , Fig. 5).

The oxidative enzyme POX was not sensitive to global-change drivers, although it was decreased by P addition in forest ( $P < 0.05$ ,

Fig. 5). Overall, the responses of EEAs to global-change drivers did not differ between forest and grassland ecosystems.

### 3.4. Detailed analysis of nitrogen-addition treatment

Among the seven global-change drivers in this study, N addition had the most data ( $> 100$ ), which allowed us to do a detailed analysis of the effect of N addition on EEAs (Fig. 6) and soil properties (Fig. S1). Therefore, we compared the responses of four representative enzymes (BG, NAG, AP and POX) and soil properties to N addition among ecosystem types, fertilizer types, application rates ( $\text{kg N ha}^{-1} \text{ yr}^{-1}$ ), and treatment durations (years).

Among the five ecosystems, forest, grassland, tundra and desert showed no significant differences (the 95% CIs of effect size overlap) in the responses of EEAs to global-change drivers, although forest generally showed the most statistically significant ( $P < 0.05$ ) responses, likely due to its largest sample size (Fig. 6). In contrast, wetland was most sensitive to N addition ( $P < 0.05$ , Fig. 6), which may be uncertain due to the small sample size.

Among the fertilizer types,  $\text{NH}_4\text{NO}_3$  was the most widely-used fertilizer, followed by urea,  $\text{NO}_3^-$  and  $\text{NH}_4^+$ . Overall, the responses of EEAs to N addition did not differ dramatically among the four fertilizer types.  $\text{NH}_4\text{NO}_3$  addition seemed to have the most significant effect on EEA (stimulation of BG and AP activity), likely due to the largest sample size. Ammonium ( $\text{NH}_4^+$ ) addition suppressed POX activity and had no significant effect on AP activity, which was different with the results of other three fertilizer types (Fig. 6).

Among the three rates of N addition (low, mid and high), low- and mid-level N addition significantly stimulated BG activity, none had significant effect on NAG activity, only mid-level N addition significantly stimulated AP activity, and only high-level N addition significantly suppressed POX activity (Fig. 6). Further analyses using the continuous randomized effects model (Table S3) indicated that the rate of N addition was significantly negatively related to the effect size of POX activity ( $P < 0.05$ ).

Among the three durations of N addition (short, mid and long), mid- and long-term N addition significantly stimulated BG activity, none had significant effect on NAG activity, short- and mid-term N addition significantly stimulated AP activity, and only short-term N addition significantly suppressed POX activity (Fig. 6). Further analyses using the continuous randomized effects model (Table S3) indicated that the duration of N addition had significant positive correlation with the effect size of BG activity but negative correlation with the effect size of POX activity ( $P < 0.05$ ).

## 4. Discussion

This meta-analysis is a comprehensive global synthesis of the responses of soil EEAs in natural ecosystems to global-change drivers in field manipulation experiments. We had hypothesized that elevated  $\text{CO}_2$  would enhance microbial enzyme activity due to the elevated input of plant-derived litter and exudates (functioning as complex organic substrate), while N and P addition (functioning as simple inorganic substrate) would suppress the enzymes acquiring the nutrient and enhance the enzymes acquiring other nutrients. In fact, our results showed that elevated  $\text{CO}_2$  had no significant effect on soil enzymes, which is not consistent with the hypothesis. In contrast, N addition enhanced C- and P-acquisition enzymes and suppressed oxidase, P addition suppressed P-acquisition AP activity, and NP addition enhanced C-acquisition enzymes, all largely consistent with the hypothesis. Additionally, we had hypothesized that warming and adding precipitation could increase EEAs, while removing precipitation could decrease EEAs. We found that decrease in precipitation had negative effect on EEAs (only significant for oxidase), increase in precipitation had positive, but insignificant, effect on EEAs, while warming suppressed oxidative activity and had weak positive effect on hydrolytic enzymes. Collectively, our results

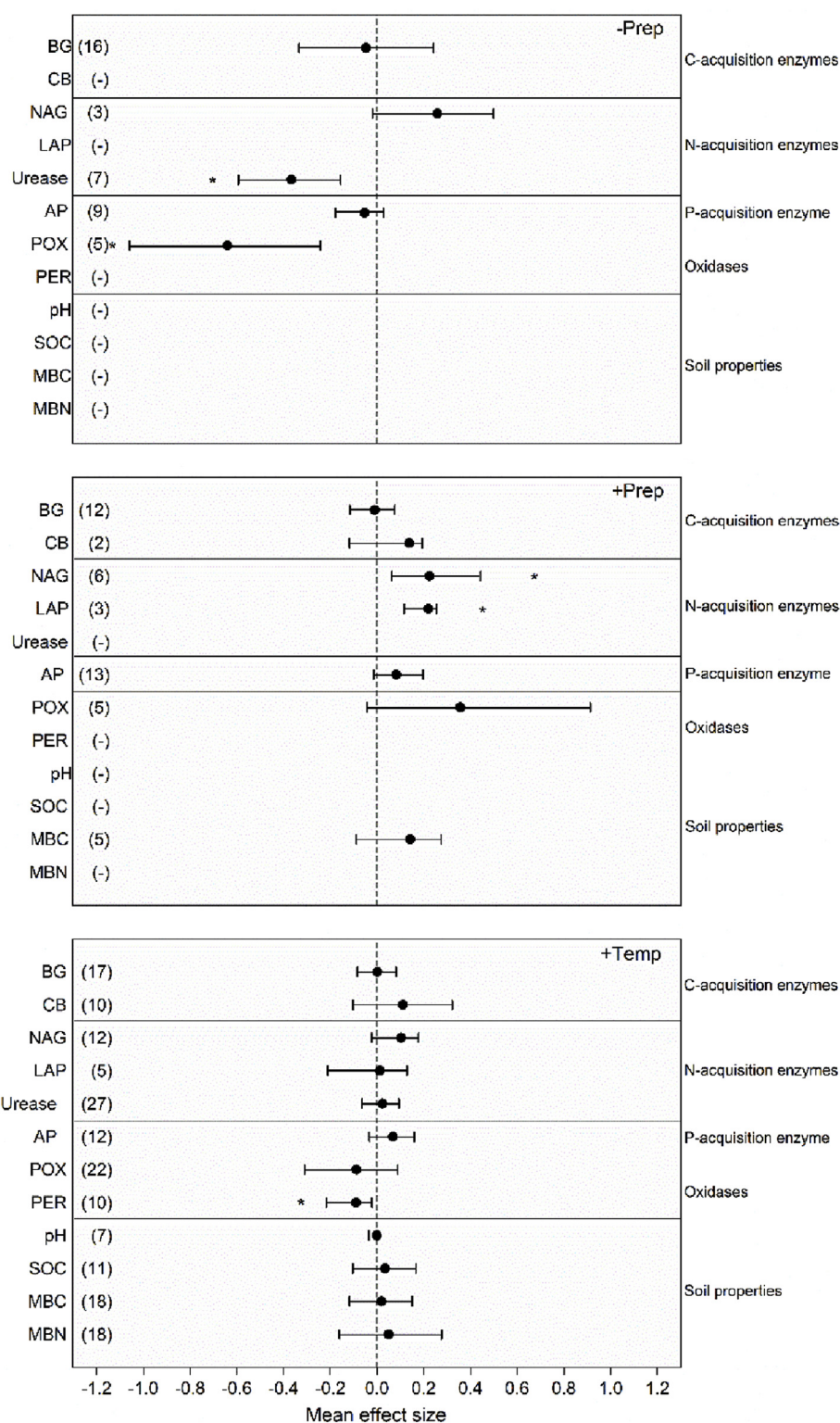


**Fig. 3.** Mean effect sizes of three global-change treatments (decrease and increase in precipitation and increase in temperature) on eight soil enzymes and four soil properties. The number in parentheses represents the sample size for each variable, and error bars represent 95% confidence intervals (CIs). \* means the mean effect size is significantly different with zero ( $P < 0.05$ ).

provide some evidence for the resource allocation theory of microbial enzyme production, and indicate that EEAs are generally more sensitive to nutrient addition than to atmospheric and climate change.

#### 4.1. Effects of nutrient addition on enzymes

In this study, we failed to detect significant effect of elevated  $\text{CO}_2$  on EEAs, although C- and N-acquisition enzymes showed positive trend with elevated  $\text{CO}_2$ . This result is not consistent with our original hypothesis, but supports an earlier meta-analysis (Kelley et al., 2011) that

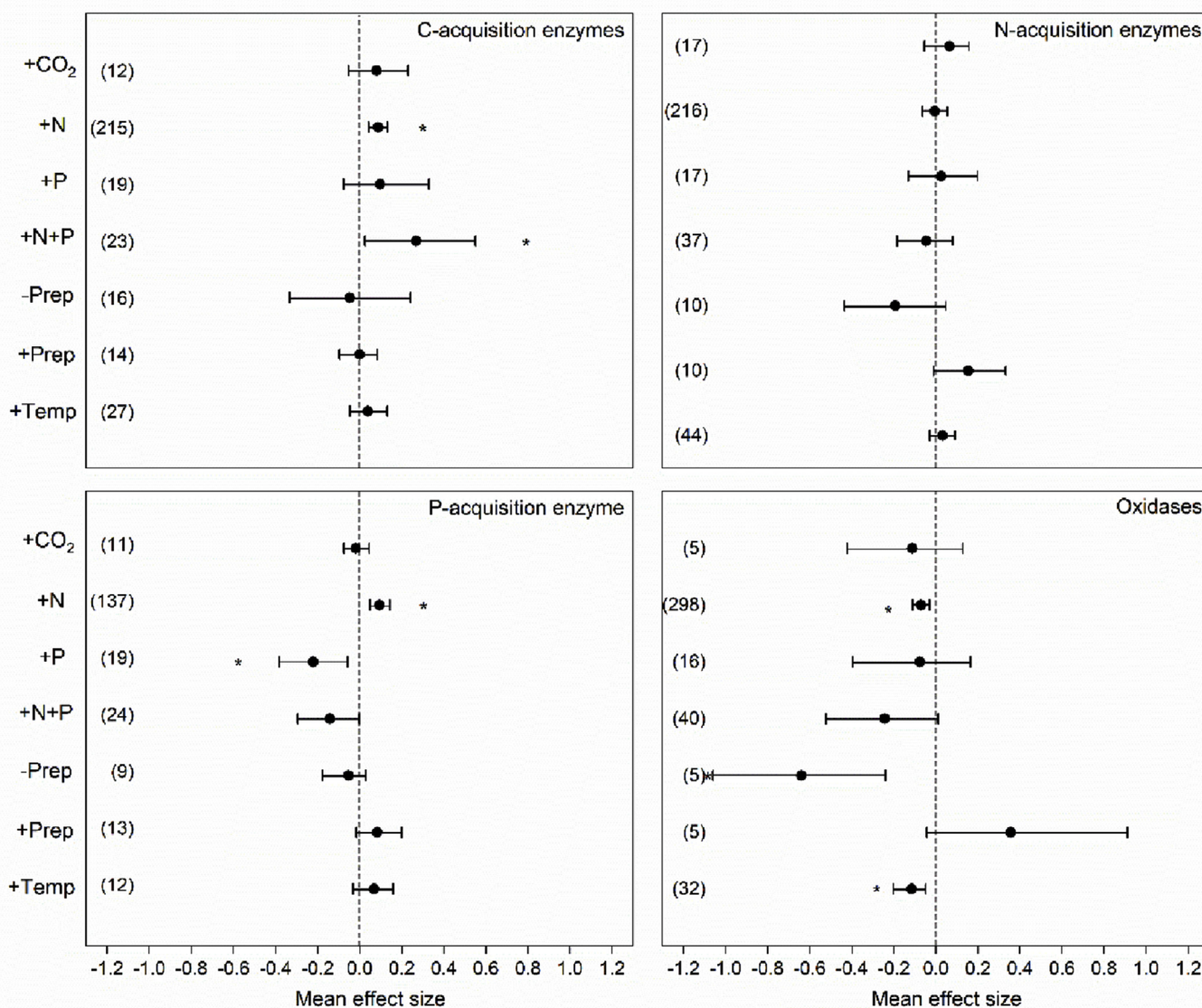


**Fig. 4.** Mean effect sizes of all seven global-change treatments (C, N, P, precipitation and temperature manipulation) on C-acquisition (BG and CB), N-acquisition (NAG, LAP and Urease), and P-acquisition (AP) and oxidative (POX and PER) enzymes. The number in parentheses represents the sample size for each variable, and error bars represent 95% confidence intervals (CIs). \* means the mean effect size is significantly different with zero ( $P < 0.05$ ).

used data from both field and greenhouse experiments. The widely different settings (soil property, environmental condition, experimental duration) in the studies of elevated CO<sub>2</sub> field experiments may prevent us from detecting statistically significant responses of soil enzymes. Despite the overall insignificant results, some trends are worthy of further discussion. For example, elevated CO<sub>2</sub> increased NAG activity by 12.9% but had no effect on AP activity. Many studies, particularly in the N-limited temperate forest and grassland ecosystems, have showed that elevated CO<sub>2</sub> may increase plant inputs (particularly root exudation and turnover) to soil, which leads to higher N-acquisition enzyme (e.g. NAG) activity and faster N turnover rate, thus meeting the plant demand of nitrogen (Kandeler et al., 2006; Phillips et al., 2011; Meier et al., 2015). Moreover, most of the elevated CO<sub>2</sub> field experiments were conducted in ecosystems not limited by P (Norby et al., 2016), which may explain the minor response of AP activity to elevated CO<sub>2</sub> in this synthesis. Notably, a recent elevated CO<sub>2</sub> experiment in P-limited mature eucalypt woodland in Australia showed that N- and P-acquisition enzymes were unexpectedly not affected by elevated CO<sub>2</sub> following 17 months of treatment (Ochoa-Hueso et al., 2017).

In contrast to the insignificant effect of elevated CO<sub>2</sub> on EEAs, we

found that N addition had mostly significant impact on EEAs (except N-acquisition hydrolytic enzyme). Specifically, N addition enhanced C- and P-acquisition enzymes, but suppressed oxidative enzymes. The result from this updated synthesis supports recent meta-analysis studies (Marklein and Houlton, 2012; Jian et al., 2016; Chen et al., 2017), and provides strong evidence for the resource allocation theory of enzyme production (Sinsabaugh and Moorhead, 1994; Allison and Vitousek, 2005) which predicted that simple, inorganic nutrient addition would suppress the activities of related nutrient-acquisition enzymes and increase the activities of other enzymes. The overall positive effect on C-acquisition (particularly BG and P-acquisition (AP) hydrolytic enzymes suggest that N addition may induce microbial demand for C and P, and thus production of enzymes to hydrolyze SOM for C and P. This is consistent with the idea that N inputs accelerate P cycling rate (Marklein and Houlton, 2012; Deng et al., 2017) and stimulate microbial decomposition of labile C-rich SOM (Cusack et al., 2011; Cenini et al., 2015). However, N addition may suppress the activity of saprotrophic fungi that produce oxidative enzymes to decompose recalcitrant SOM, which further leads to increase in soil carbon storage, particularly in forest ecosystems with high-lignin, low-quality litter (Waldrop et al.,

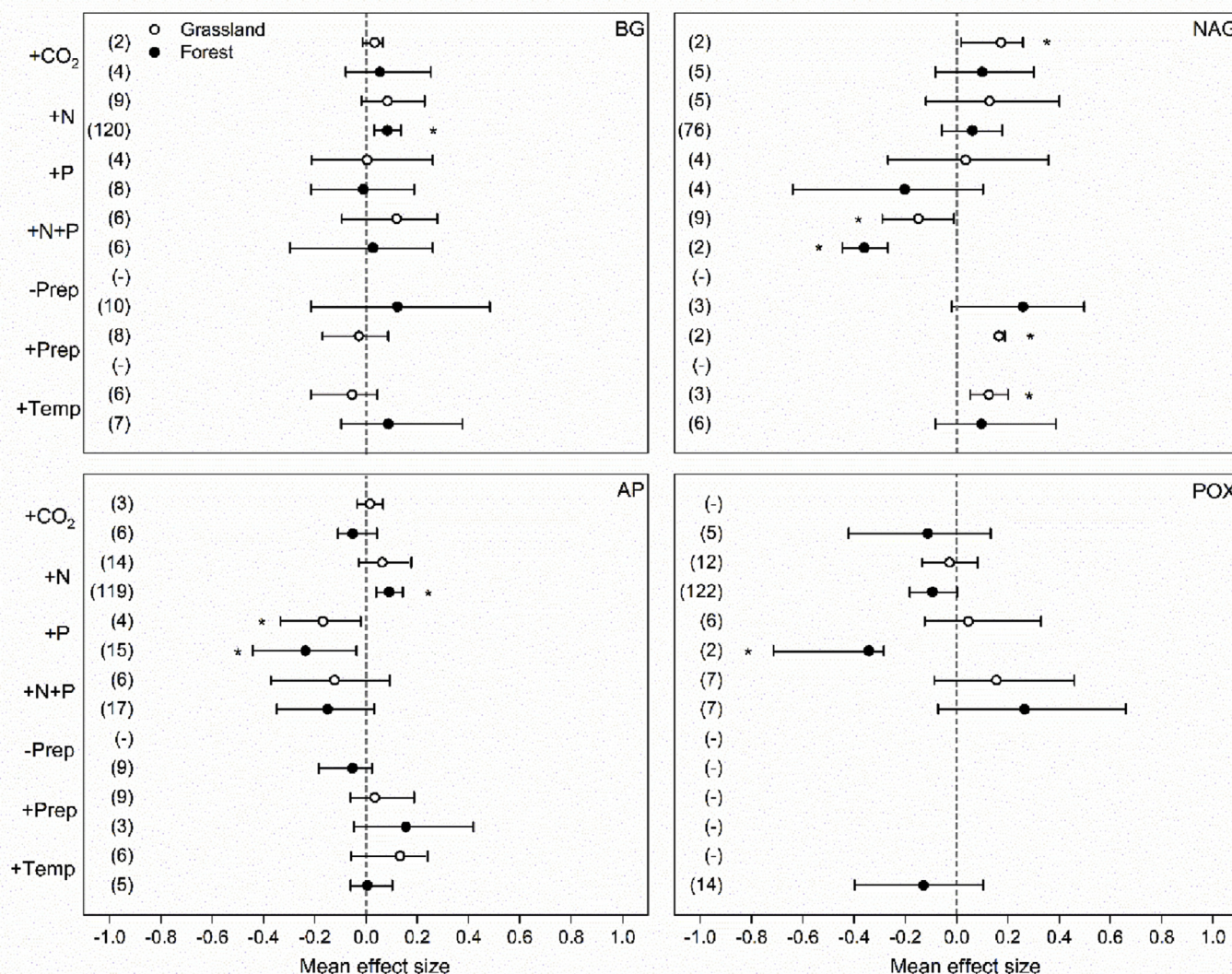


Fig. 5. Comparison of mean effect sizes of all seven global-change treatments (C, N, P, precipitation and temperature manipulation) on three hydrolytic enzymes related to C (BG), N (NAG), and P (AP) acquisition and an oxidative enzyme (POX) between grassland (empty circles) and forest (solid circles) ecosystems. The number in parentheses represents the sample size for each variable, and error bars represent 95% confidence intervals (CIs). \* means the mean effect size is significantly different with zero ( $P < 0.05$ ).

2004; Cusack et al., 2011). Surprisingly, N addition had minor effect on N-acquisition hydrolytic enzymes, but N addition may still suppress overall activity of N-acquisition enzymes as oxidase can mine N from recalcitrant SOM (Sinsabaugh, 2010; Zhu et al., 2014). In addition, N addition led to soil acidification and decrease in the C:N ratio of microbial biomass (increase in MBN but decrease in MBC, Fig. 2), indicating a shift toward more bacteria-dominated copiotrophic microbial community (Strickland and Rousk, 2010; Leff et al., 2015), which may contribute to change in microbial enzyme activities and increase in soil carbon storage (Cusack et al., 2011).

Phosphorus addition suppressed AP activity by  $-20\%$ , with no significant effect on other enzymes. This finding supports an earlier meta-analysis that P addition suppresses root and soil phosphatase activity (Marklein and Houlton, 2012), which is also found in a few recent P fertilization experiments in tropical forests (Zheng et al., 2015; Yokoyama et al., 2017). Additionally, combined N and P addition led to dramatic increase in C-acquisition hydrolytic enzyme activity (particularly BG), suggesting that NP addition stimulates microbial decomposition of labile SOM. Although combined NP addition tended to suppress N- and P-acquisition enzymes, the effect was insignificant and enzyme-specific, likely due to the cancelling effect of N vs. P addition

on these enzymes. It is noteworthy that NP addition increased SOC by  $8.4\%$ , which may partly be caused by the reduction in oxidative enzymes that degrade recalcitrant SOM.

#### 4.2. Effects of climatic change on enzymes

The impact of altered precipitation (increase or decrease) on soil EEAs are mostly not significant, likely due to the small sample size ( $n < 16$ ). Despite this limitation, decrease in precipitation (drying) tends to suppress soil EEAs, particularly oxidative enzymes, while increase in precipitation (watering) tends to enhance soil EEAs, particularly N-acquisition hydrolytic and oxidative enzymes (NAG, LAP, POX). This general pattern is consistent with our hypothesis that wetter soils have higher microbial enzyme activity than drier soils (Henry, 2012). Despite this general pattern, the variation among different studies was large, which led to statistically insignificant effect sizes for the water manipulation experiments. The context dependence of these results may partly be explained by the variation in soil properties, such as the initial soil moisture in the control and manipulated treatments. For example, water drawdown in water-saturated anaerobic soil can increase oxygen content, which stimulates soil EEAs, while further drying

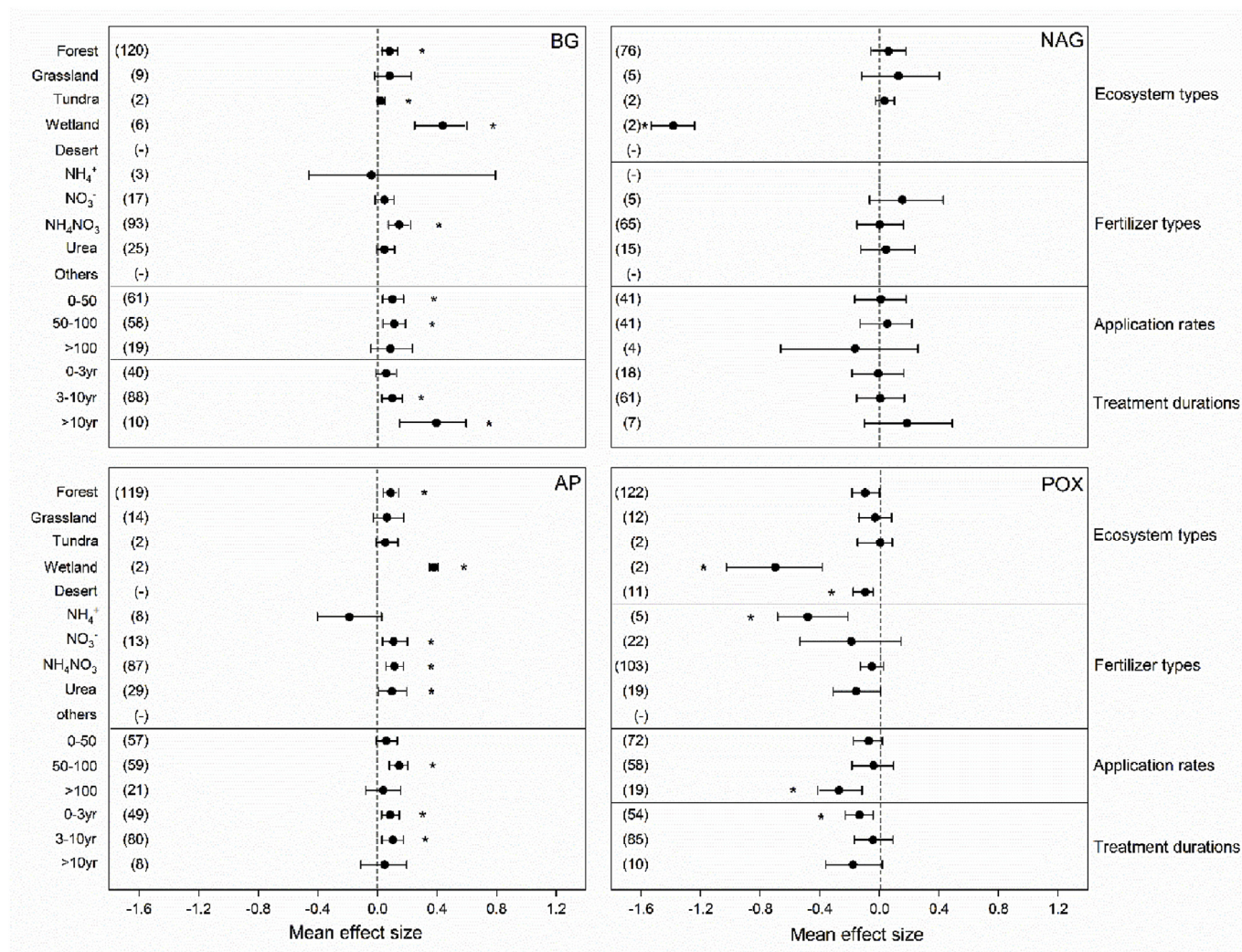


Fig. 6. Comparison of mean effect sizes of nitrogen addition on three hydrolytic enzymes related to C (BG), N (NAG), and P (AP) acquisition and an oxidative enzyme (POX) among ecosystem types, fertilizer types, application rates ( $\text{kg N ha}^{-1} \text{yr}^{-1}$ ), and treatment durations (yrs). The number in parentheses represents the sample size for each variable, and error bars represent 95% confidence intervals (CIs). \* means the mean effect size is significantly different with zero ( $P < 0.05$ ).

can suppress soil EEAs by increasing water limitation for substrate diffusion (Henry, 2012). Therefore, inconsistent responses of soil EEAs to water manipulation are often observed. For example, high-precipitation treatment showed higher (Sardans and Penuelas, 2005), lower (Gutknecht et al., 2010), and similar (McDaniel et al., 2013) soil EEAs compared to the control in various field experiments.

Compared to altered precipitation, warming has smaller effects soil EEAs. Our meta-analysis demonstrated that warming only significantly suppressed oxidase activity, and had insignificant positive effect on hydrolytic enzymes. On one hand, warming can increase plant substrate input and soil microbial activity, which further leads to faster soil C and N cycling rates (Bai et al., 2013; Lu et al., 2013). On the other hand, warming can also increase plant transpiration and reduce soil moisture, which can suppress microbial enzyme activities (Allison and Treseder, 2008; Henry, 2012). These positive and negative effects of warming on microbial enzyme activity are driven by both direct and indirect effects on various plant and soil variables, and can cancel each other out, leading to overall weak or neutral effects on soil EEAs. For example, a number of field experiments have found minor responses of soil EEAs to warming in forest and grassland ecosystems (McDaniel et al., 2013; Jing et al., 2014; Wang et al., 2014; Slaughter et al., 2015). It should be noted that the warming effect on soil EEAs varies with season and year, and the temporal variations in EEAs are often stronger than variations

caused by climate manipulation in field experiments (Bell et al., 2010; Slaughter et al., 2015).

Importantly, the incubation temperature for enzyme assay in the lab varied greatly in field-warming studies and also differed significantly from the soil temperature of control or warming treatments. For example, some studies (Steinweg et al., 2013) used room temperature (varying from 20 to 25 °C), some studies (Sardans et al., 2008) used higher temperature (37 °C), and other studies used lower temperature (e.g., 10 °C), approximately the average soil temperature during the summertime in all the treatments (Dominguez et al., 2017) or did not provide the temperature data for enzyme assay. As a notable exception, a recent study (Souza et al., 2017) used various temperatures for enzyme assay – mean temperature for both control and warming treatments during the growing season, 10 and 14 °C, respectively, and an optimum temperature for enzyme activity, 25 °C. As enzyme activity mostly increases with incubation temperature (e.g., Jing et al., 2014; Souza et al., 2017), use of common incubation temperature for both control and warming treatments (e.g., 25 °C) in those field-warming studies may not reflect the temperature sensitivity of enzymes, and likely underestimate the impact of warming on *in situ* enzyme activity.

#### 4.3. No significant difference between forest and grassland

We further investigated whether the responses of EEAs to global-change drivers vary between the two major ecosystems in our database – forest and grassland. Overall, forest and grassland showed no meaningful differences in the responses of EEAs to global-change drivers. Strikingly, both magnitude and direction of the responses are very similar between the two ecosystems, despite distinct vegetation characteristics (trees vs. grasses), soil properties (pH, nutrient availability), and microbial communities (fungi vs. bacteria) between forest and grassland. This overall pattern is unexpected given the often ecosystem-specific responses of EEA to global-change drivers (Waldrop et al., 2004; Keeler et al., 2009). However, a few enzymes did show stronger responses in forest than in grassland ecosystems, particularly in N-addition experiments which have the most data and the highest possibility to detect the difference. For instance, the positive effect of N addition on C-acquisition (BG) and P-acquisition (AP) enzyme activity was statistically significant in forest, but not in grassland. Additionally, the negative effect of N addition on oxidative enzyme activity was marginally significant in forest, but very weak in grassland. This contrasting result is likely because N addition suppressed oxidative enzyme activity in Basidiomycota-dominated soils in temperate and boreal forest, while had little effect in grassland soils dominated by Glomeromycota and Ascomycota (Sinsabaugh, 2010; Tedersoo et al., 2014; Leff et al., 2015; Riggs and Hobbie, 2016). Notably, the small sample size in grassland compared to forest ecosystems makes this comparison uncertain. Future studies on soil EEAs in grassland and other non-forest ecosystems (Zhou et al., 2013; Cenini et al., 2015; Jing et al., 2016; Riggs and Hobbie, 2016) are urgently needed to reduce this uncertainty.

#### 4.4. Effects of N addition on enzymes

We also did a detailed analysis of the effect of N addition on EEAs, because only N-addition experiments had enough data to compare the effect size among ecosystem types, fertilizer types, application rates, and treatment durations. Wetland soil EEAs seem to be most sensitive to N addition, but the result should be interpreted with caution given only a handful of experiments in wetland (including peatland) ecosystems (Song et al., 2013; Pinsonneault et al., 2016). The effect size in forest ecosystem was mostly significant, which is likely due to the relatively large sample size ( $n > 100$ ). Additionally, the effect of N addition on EEAs did not differ significantly among the four fertilizer types. However, only  $\text{NH}_4\text{NO}_3$  addition significantly enhanced BG activity, consistent with the finding in a recent synthesis (Jian et al., 2016), and only ammonium addition significantly suppressed POX activity, likely associated with the stronger soil acidification by ammonium than other nitrogen forms (Zhang et al., 2017). Moreover, nitrogen application rate showed significant negative correlation with the effect size of POX activity, suggesting that the suppression of POX activity produced by saprotrophic fungi increases with increasing nitrogen load (Zhou et al., 2012). Similarly, the significant correlation between the duration of N addition and the effect size of BG (positive) and POX (negative) activity respectively indicates that long-term fertilization experiments would cause more enhancement of C-acquisition enzyme and more suppression of oxidative enzyme. Such long-term experiments may be needed to detect a significant change in microbial enzyme activity and soil carbon storage (Lu et al., 2011; Zak et al., 2017).

#### 5. Conclusion

Considering the key roles soil enzymes played in litter and SOM decomposition, nitrogen and phosphorus mineralization, EEAs have been increasingly incorporated into ecosystem and earth system models to predict the response of soil carbon storage to global change (Wieder et al., 2015). This comprehensive meta-analysis quantitatively synthesized the responses of common soil enzymes to multiple global-change

drivers based on ecosystem-level field manipulation experiments. We showed that soil EEAs are more sensitive to changes in nutrient availability caused by N and P deposition than to changes in atmospheric  $\text{CO}_2$ , temperature and precipitation. Particularly, N and P addition significantly affect soil EEAs which is largely consistent with the resource allocation theory, warming and precipitation increase tend to accelerate but drought tends to suppress soil carbon and nutrient cycling, and elevated  $\text{CO}_2$  only has minor effect on soil enzyme activities. Such patterns are not entirely consistent with predictions in enzyme-based models, likely due to the small number of observations from field experiments for some global-change drivers (e.g., elevated  $\text{CO}_2$  and climate change). Given that the current methods of soil enzyme assays have limitations in measuring *in situ* activity (Nannipieri et al., 2012; Burns et al., 2013), future studies should aim to improve the method of enzyme assay, such as standard protocol for microplate enzyme assay (Dick et al., 2018), soil zymography for *in situ* mapping of enzyme spatial distribution (Spohn et al., 2013), and enzyme-coding fictional gene analysis (Nannipieri et al., 2017). Moreover, future studies should collect more data from ecosystems (e.g., wetland, tundra and desert, particularly in the southern hemisphere) or global-change drivers (e.g., elevated  $\text{CO}_2$ , warming, and altered precipitation) which currently lack enzyme data. Nevertheless, our global synthesis of the responses of soil enzyme activities to global-change drivers can be used to explicitly represent microbial processes into ecosystem and earth system models.

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#### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2018.05.001>.

#### References

- Adams, D.C., Gurevitch, J., Rosenberg, M.S., 1997. Resampling tests for meta-analysis of ecological data. *Ecology* 78, 1277–1283.
- Allison, S.D., Czimczik, C.L., Treseder, K.K., 2008. Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. *Global Change Biology* 14, 1156–1168.
- Allison, S.D., Treseder, K.K., 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. *Global Change Biology* 14, 2898–2909.
- Allison, S.D., Vitousek, P.M., 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biology and Biochemistry* 37, 937–944.
- Bai, E., Li, S., Xu, W., Li, W., Dai, W., Jiang, P., 2013. A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics. *New Phytologist* 199, 441–451.
- Bell, T.H., Klironomos, J.N., Henry, H.A.L., 2010. Seasonal responses of extracellular enzyme activity and microbial biomass to warming and nitrogen addition. *Soil Science Society of America Journal* 74, 820–828.
- Brockett, B.F.T., Prescott, C.E., Grayston, S.J., 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. *Soil Biology and Biochemistry* 44, 9–20.
- Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: current knowledge and future directions. *Soil Biology and Biochemistry* 58, 216–234.
- Cenini, V.L., Fornara, D.A., McMullan, G., Ternan, N., Lajtha, K., Crawley, M.J., 2015. Chronic nitrogen fertilization and carbon sequestration in grassland soils: evidence of a microbial enzyme link. *Biogeochemistry* 126, 301–313.
- Chen, J., Luo, Y., Li, J., Zhou, X., Cao, J., Wang, R.-W., Wang, Y., Shelton, S., Jin, Z., Walker, L.M., Feng, Z., Niu, S., Feng, W., Jian, S., Zhou, L., 2017. Costimulation of soil glycosidase activity and soil respiration by nitrogen addition. *Global Change Biology* 23, 1328–1337.
- Cusack, D.F., Silver, W.L., Torn, M.S., Burton, S.D., Firestone, M.K., 2011. Changes in microbial community characteristics and soil organic matter with nitrogen additions in two tropical forests. *Ecology* 92, 621–632.

- Deng, Q., Hui, D., Dennis, S., Reddy, K.C., 2017. Responses of terrestrial ecosystem phosphorus cycling to nitrogen addition: a meta-analysis. *Global Ecology and Biogeography* 26, 713–728.
- Dick, R.P., Dick, L.K., Deng, S., Li, X., Kandler, E., Poll, C., Freeman, C., Jones, T.G., Weintraub, M.N., Eselli, K.A., Saxena, J., 2018. Cross-laboratory comparison of fluorimetric microplate and colorimetric bench-scale soil enzyme assays. *Soil Biology and Biochemistry* 121, 240–248.
- Dieleman, W.I.J., Janssens, I.A., 2011. Can publication bias affect ecological research? A case study on soil respiration under elevated CO<sub>2</sub>. *New Phytologist* 190, 517–521.
- Dominguez, M.T., Holthof, E., Smith, A.R., Koller, E., Emmett, B.A., 2017. Contrasting response of summer soil respiration and enzyme activities to long-term warming and drought in a wet shrubland (NE Wales, UK). *Applied Soil Ecology* 110, 151–155.
- Dong, W.Y., Zhang, X.Y., Liu, X.Y., Fu, X.L., Chen, F.S., Wang, H.M., Sun, X.M., Wen, X.F., 2015. Responses of soil microbial communities and enzyme activities to nitrogen and phosphorus additions in Chinese fir plantations of subtropical China. *Biogeosciences* 12, 5537–5546.
- Finzi, A.C., Sinsabaugh, R.L., Long, T.M., Osgood, M.P., 2006. Microbial community responses to atmospheric carbon dioxide enrichment in a warm-temperate forest. *Ecosystems* 9, 215–226.
- Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., Vorosmarty, C.J., 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry* 70, 153–226.
- Gutknecht, J.L.M., Henry, H.A.L., Balser, T.C., 2010. Inter-annual variation in soil extracellular enzyme activity in response to simulated global change and fire disturbance. *Pedobiologia* 53, 283–293.
- Hedges, L.V., Gurevitch, J., Curtis, P.S., 1999. The meta-analysis of response ratios in experimental ecology. *Ecology* 80, 1150–1156.
- Henry, H.A.L., 2012. Soil extracellular enzyme dynamics in a changing climate. *Soil Biology and Biochemistry* 47, 53–59.
- Henry, H.A.L., Juarez, J.D., Field, C.B., Vitousek, P.M., 2005. Interactive effects of elevated CO<sub>2</sub>, N deposition and climate change on extracellular enzyme activity and soil density fractionation in a California annual grassland. *Global Change Biology* 11, 1808–1815.
- Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J.A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A.J., Grace, J., Matteucci, G., Papale, D., Piao, S.L., Schulze, E.D., Tang, J., Law, B.E., 2010. Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience* 3, 315–322.
- Jian, S., Li, J., Chen, J., Wang, G., Mayes, M.A., Dzanter, K.E., Hui, D., Luo, Y., 2016. Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: a meta-analysis. *Soil Biology and Biochemistry* 101, 32–43.
- Jing, X., Chen, X., Tang, M., Ding, Z., Jiang, L., Li, P., Ma, S., Tian, D., Xu, L., Zhu, J., Ji, C., Shen, H., Zheng, C., Fang, J., Zhu, B., 2017. Nitrogen deposition has minor effect on soil extracellular enzyme activities in six Chinese forests. *The Science of the Total Environment* 607, 806–815.
- Jing, X., Wang, Y., Chung, H., Mi, Z., Wang, S., Zeng, H., He, J.-S., 2014. No temperature acclimation of soil extracellular enzymes to experimental warming in an alpine grassland ecosystem on the Tibetan Plateau. *Biogeochemistry* 117, 39–54.
- Jing, X., Yang, X., Ren, F., Zhou, H., Zhu, B., He, J.-S., 2016. Neutral effect of nitrogen addition and negative effect of phosphorus addition on topsoil extracellular enzymatic activities in an alpine grassland ecosystem. *Applied Soil Ecology* 107, 205–213.
- Kandler, E., Mosier, A.R., Morgan, J.A., Milchunas, D.G., King, J.Y., Rudolph, S., Tschirko, D., 2006. Response of soil microbial biomass and enzyme activities to the transient elevation of carbon dioxide in a semi-arid grassland. *Soil Biology and Biochemistry* 38, 2448–2460.
- Keeler, B.L., Hobbie, S.E., Kellogg, L.E., 2009. Effects of long-term nitrogen addition on microbial enzyme activity in eight forested and grassland sites: implications for litter and soil organic matter decomposition. *Ecosystems* 12, 1–15.
- Kelley, A.M., Pay, P.A., Polley, H.W., Gill, R.A., Jackson, R.B., 2011. Atmospheric CO<sub>2</sub> and soil extracellular enzyme activity: a meta-analysis and CO<sub>2</sub> gradient experiment. *Ecosphere* 2.
- Kreyling, J., Beierkuhnlein, C., Elmer, M., Pritsch, K., Radovsky, M., Schloter, M., Woelcke, J., Jentsch, A., 2008. Soil biotic processes remain remarkably stable after 100-year extreme weather events in experimental grassland and heath. *Plant and Soil* 308, 175–188.
- Leff, J.W., Jones, S.E., Prober, S.M., Barberan, A., Borer, E.T., Firn, J.L., Harpole, W.S., Hobbie, S.E., Hofmockel, K.S., Knops, J.M.H., McCulley, R.L., La Pierre, K., Risch, A.C., Seabloom, E.W., Schuetz, M., Steenbock, C., Stevens, C.J., Fierer, N., 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the National Academy of Sciences of the United States of America* 112, 10967–10972.
- Lu, M., Zhou, X., Luo, Y., Yang, Y., Fang, C., Chen, J., Li, B., 2011. Minor stimulation of soil carbon storage by nitrogen addition: a meta-analysis. *Agriculture, Ecosystems & Environment* 140, 234–244.
- Lu, M., Zhou, X., Yang, Q., Li, H., Luo, Y., Fang, C., Chen, J., Yang, X., Li, B., 2013. Responses of ecosystem carbon cycle to experimental warming: a meta-analysis. *Ecology* 94, 726–738.
- Marklein, A.R., Houlton, B.Z., 2012. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytologist* 193, 696–704.
- McDaniel, M.D., Kaye, J.P., Kaye, M.W., 2013. Increased temperature and precipitation had limited effects on soil extracellular enzyme activities in a post-harvest forest. *Soil Biology and Biochemistry* 56, 90–98.
- Meier, I.C., Pritchard, S.G., Brzostek, E.R., McCormack, M.L., Phillips, R.P., 2015. The rhizosphere and hyphosphere differ in their impacts on carbon and nitrogen cycling in forests exposed to elevated CO<sub>2</sub>. *New Phytologist* 205, 1164–1174.
- Nannipieri, P., Giagnoni, L., Renella, G., Puglisi, E., Ceccanti, B., Masciandaro, G., Fornasier, F., Moscatelli, M.C., Marinari, S., 2012. Soil enzymology: classical and molecular approaches. *Biology and Fertility of Soils* 48, 743–762.
- Nannipieri, P., Trasar-Cepeda, C., Dick, R.P., 2017. Soil enzyme activity: a brief history and biochemistry as a basis for appropriate interpretations and meta-analysis. *Biology and Fertility of Soils* 54, 11–19.
- Norby, R.J., De Kauwe, M.G., Domingues, T.F., Duursma, R.A., Ellsworth, D.S., Goll, D.S., Lapola, D.M., Luus, K.A., MacKenzie, A.R., Medlyn, B.E., Pavlick, R., Rammig, A., Smith, B., Thomas, R., Thonicke, K., Walker, A.P., Yang, X., Zaehle, S., 2016. Model-data synthesis for the next generation of forest free-air CO<sub>2</sub> enrichment (FACE) experiments. *New Phytologist* 209, 17–28.
- Ochoa-Hueso, R., Hughes, J., Delgado-Baquerizo, M., Drake, J.E., Tjoelker, M.G., Pineiro, J., Power, S.A., 2017. Rhizosphere-driven increase in nitrogen and phosphorus availability under elevated atmospheric CO<sub>2</sub> in a mature Eucalyptus woodland. *Plant and Soil* 416, 283–295.
- Phillips, R.P., Finzi, A.C., Bernhardt, E.S., 2011. Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO<sub>2</sub> fumigation. *Ecology Letters* 14, 187–194.
- Pinsonneault, A.J., Moore, T.R., Roulet, N.T., 2016. Effects of long-term fertilization on peat stoichiometry and associated microbial enzyme activity in an ombrotrophic bog. *Biogeochemistry* 129, 149–164.
- Ren, C., Zhao, F., Shi, Z., Chen, J., Han, X., Yang, G., Feng, Y., Ren, G., 2017. Differential responses of soil microbial biomass and carbon-degrading enzyme activities to altered precipitation. *Soil Biology and Biochemistry* 115, 1–10.
- Riggs, C.E., Hobbie, S.E., 2016. Mechanisms driving the soil organic matter decomposition response to nitrogen enrichment in grassland soils. *Soil Biology and Biochemistry* 99, 54–65.
- Rosenberg, M.S., Adams, D.C., Gurevitch, J., 2000. *MetaWin: Statistical Software for Meta-analysis*. Sinauer Associates Sunderland.
- Saiya-Cork, K.R., Sinsabaugh, R.L., Zak, D.R., 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. *Soil Biology and Biochemistry* 34, 1309–1315.
- Sardans, J., Penuelas, J., 2005. Drought decreases soil enzyme activity in a Mediterranean *Quercus ilex* L. forest. *Soil Biology and Biochemistry* 37, 455–461.
- Sardans, J., Penuelas, J., 2010. Soil enzyme activity in a mediterranean forest after six years of drought. *Soil Science Society of America Journal* 74, 838–851.
- Sardans, J., Penuelas, J., Estiarte, M., 2008. Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. *Applied Soil Ecology* 39, 223–235.
- Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biology and Biochemistry* 42, 391–404.
- Sinsabaugh, R.L., Moorhead, D.L., 1994. Resource-allocation to extracellular enzyme production-A model for nitrogen and phosphorus control of litter. *Soil Biology and Biochemistry* 26, 1305–1311.
- Slaughter, L.C., Weintraub, M.N., McCulley, R.L., 2015. Seasonal effects stronger than three-year climate manipulation on grassland soil microbial community. *Soil Science Society of America Journal* 79, 1352–1365.
- Song, Y., Song, C., Li, Y., Hou, C., Yang, G., Zhu, X., 2013. Short-term effect of nitrogen addition on litter and soil properties in *calamagrostis angustifolia* freshwater marshes of Northeast China. *Wetlands* 33, 505–513.
- Souza, R.C., Solly, E.F., Dawes, M.A., Graf, F., Hagedorn, F., Egli, S., Clement, C.R., Nagy, L., Rixen, C., Peter, M., 2017. Responses of soil extracellular enzyme activities to experimental warming and CO<sub>2</sub> enrichment at the alpine treeline. *Plant and Soil* 416, 527–537.
- Spohn, M., Carminati, A., Kuzyakov, Y., 2013. Soil zymography – a novel in situ method for mapping distribution of enzyme activity in soil. *Soil Biology and Biochemistry* 58, 275–280.
- Steinweg, J.M., Dukes, J.S., Paul, E.A., Wallenstein, M.D., 2013. Microbial responses to multi-factor climate change: effects on soil enzymes. *Frontiers in Microbiology* 4.
- Stone, M.M., Plante, A.F., Casper, B.B., 2013. Plant and nutrient controls on microbial functional characteristics in a tropical Oxisol. *Plant and Soil* 373, 893–905.
- Strickland, M.S., Rousk, J., 2010. Considering fungal:bacterial dominance in soils – methods, controls, and ecosystem implications. *Soil Biology and Biochemistry* 42, 1385–1395.
- Tedersoo, L., Bahram, M., Polme, S., Koljalg, U., Yorou, N.S., Wijesundera, R., Villarreal Ruiz, L., Vasco-Palacios, A.M., Pham Quang, T., Suija, A., Smith, M.E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Poldmaa, K., Piepenbring, M., Phosri, C., Peterson, M., Parts, K., Paertel, K., Otsing, E., Nohra, E., Njouonkou, A.L., Nilsson, R.H., Morgado, L.N., Mayor, J., May, T.W., Majuakim, L., Lodge, D.J., Lee, S.S., Larsson, K.-H., Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T.W., Harend, H., Guo, L.-d., Greslebin, A., Grelet, G., Geml, J., Gates, G., Dunstan, W., Dunk, C., Drenkhan, R., Deamaley, J., De Kesel, A., Tan, D., Chen, X., Buegger, F., Brearley, F.Q., Bonito, G., Anslan, S., Abell, S., Abarenkov, K., 2014. Global diversity and geography of soil fungi. *Science*, vol. 346 1078–+.
- Turner, B.L., Wright, S.J., 2014. The response of microbial biomass and hydrolytic enzymes to a decade of nitrogen, phosphorus, and potassium addition in a lowland tropical rain forest. *Biogeochemistry* 117, 115–130.
- Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., Gallo, M., Lauber, C., 2004. Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. *Ecological Applications* 14, 1172–1177.
- Wang, X., Dong, S., Gao, Q., Zhou, H., Liu, S., Su, X., Li, Y., 2014. Effects of short-term and long-term warming on soil nutrients, microbial biomass and enzyme activities in an alpine meadow on the Qinghai-Tibet Plateau of China. *Soil Biology and Biochemistry* 76, 140–142.
- Weedon, J.T., Aerts, R., Kowalchuk, G.A., van Bodegom, P.M., 2011. Enzymology under global change: organic nitrogen turnover in alpine and sub-Arctic soils. *Biochemical Society Transactions* 39, 309–314.

- Wieder, W.R., Allison, S.D., Davidson, E.A., Georgiou, K., Hararuk, O., He, Y., Hopkins, F., Luo, Y., Smith, M.J., Sulman, B., Todd-Brown, K., Wang, Y.-P., Xia, J., Xu, X., 2015. Explicitly representing soil microbial processes in Earth system models. *Global Biogeochemical Cycles* 29, 1782–1800.
- Yokoyama, D., Imai, N., Kitayama, K., 2017. Effects of nitrogen and phosphorus fertilization on the activities of four different classes of fine-root and soil phosphatases in Bornean tropical rain forests. *Plant and Soil* 416, 463–476.
- Zak, D.R., Freedman, Z.B., Upchurch, R.A., Steffens, M., Koegel-Knabner, I., 2017. Anthropogenic N deposition increases soil organic matter accumulation without altering its biochemical composition. *Global Change Biology* 23, 933–944.
- Zeglin, L.H., Stursova, M., Sinsabaugh, R.L., Collins, S.L., 2007. Microbial responses to nitrogen addition in three contrasting grassland ecosystems. *Oecologia* 154, 349–359.
- Zhang, C., Zhang, X.-Y., Zou, H.-T., Kou, L., Yang, Y., Wen, X.-F., Li, S.-G., Wang, H.-M., Sun, X.-M., 2017. Contrasting effects of ammonium and nitrate additions on the biomass of soil microbial communities and enzyme activities in subtropical China. *Biogeosciences* 14, 4815–4827.
- Zheng, M., Huang, J., Chen, H., Wang, H., Mo, J., 2015. Responses of soil acid phosphatase and beta-glucosidase to nitrogen and phosphorus addition in two subtropical forests in southern China. *European Journal of Soil Biology* 68, 77–84.
- Zhou, L., Zhou, X., Zhang, B., Lu, M., Luo, Y., Liu, L., Li, B., 2014. Different responses of soil respiration and its components to nitrogen addition among biomes: a meta-analysis. *Global Change Biology* 20, 2332–2343.
- Zhou, X., Chen, C., Wang, Y., Xu, Z., Han, H., Li, L., Wan, S., 2013. Warming and increased precipitation have differential effects on soil extracellular enzyme activities in a temperate grassland. *The Science of the Total Environment* 444, 552–558.
- Zhou, X., Zhang, Y., Downing, A., 2012. Non-linear response of microbial activity across a gradient of nitrogen addition to a soil from the Gurbantunggut Desert, northwestern China. *Soil Biology and Biochemistry* 47, 67–77.
- Zhu, B., Gutknecht, J.L.M., Herman, D.J., Keck, D.C., Firestone, M.K., Cheng, W., 2014. Rhizosphere priming effects on soil carbon and nitrogen mineralization. *Soil Biology and Biochemistry* 76, 183–192.